Review Article

Experimental studies of forensic odontology to aid in the identification process

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Abstract

The importance of dental identification is on the increase year after year. With the passage of time, the role of forensic odontology has increased as very often teeth and dental restorations are the only means of identification. Forensic odontology has played a key role in identification of persons in mass disasters (aviation, earthquakes, Tsunamis), in crime investigations, in ethnic studies, and in identification of decomposed and disfigured bodies like that of drowned persons, fire victims, and victims of motor vehicle accidents. The various methods employed in forensic odontology include tooth prints, radiographs, photographic study, rugoscopy, cheiloscopy and molecular methods. Investigative methods applied in forensic odontology are reasonably reliable, yet the shortcomings must be accounted for to make it a more meaningful and relevant procedure. This paper gives an overview of the various experimental studies to aid in the identification processes, discussing their feasibilities and limitations in day-to-day practice.

Key words: Age estimation, cheiloscopy, experimental studies, forensic odontology, molecular methods, rugoscopy, sex determination, tooth prints

Introduction

Human identification is one of the most challenging subjects that man has been confronted with. The forensic discipline is concerned with the application of science and technology to the detection and investigation of crime and administration of justice, requiring the coordinated efforts of a multidisciplinary team.[1] Dental identification remains one of the most reliable and frequently applied methods of identification, predominantly by the comparisons of ante-mortem and post-mortem records.[2] The science of dealing with evidence from dental and oral structures – Forensic Odontology, is a specialty unto itself. The establishment of forensic odontology as a unique discipline has been attributed to Dr. Oscar Amoeda (Father of Forensic Odontology), who identified the victims of a fire accident in Paris, France in 1898 [Table 1].[3] This branch has an established domain with wide applications in:

- Examination and evaluation of injuries to jaws, teeth and oral soft tissues,
- Identification of individuals in criminal investigations and/or mass disasters,
- Identification, examination and evaluation of bite marks which occur with some frequency in sexual assaults, child abuse and personal defence situations, and
- Age estimation[1]

The various other methods employed in forensic odontology include rugoscopy (study of palatal rugae patterns),[4] cheiloscopy (study of lip prints),[5] tooth prints,[6] radiographs,[7] photographic study and molecular methods like polymerase chain reaction (PCR) for pulp DNA analysis.[8]

In the present era of genomics and proteomics, forensic dentistry has encompassed the use of DNA and proteins as unique tools in identification. Genomic and mitochondrial

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DNA from the pulp, dentin or cementum of teeth or desquamated cells in saliva are vital to the forensic process of identification.[9]

Dental identification takes three main forms. Firstly, the most frequently performed examination is a comparative identification that is used to establish (to a higher degree of certainty) that the remains of a decedent and a person represented by ante-mortem dental records are of the same individual. Secondly, in those cases where ante-mortem records are not available and no clues to the possible identity exist, a post-mortem dental profile is completed by the forensic dentist suggesting characteristics of the individual likely to narrow the search for the ante-mortem materials, this is referred to as dental profiling and thirdly, forensic odontologists also play a vital role in identification of victims of mass disasters.[10,11]

Studies for Age Estimation and Sex Determination

Mandibular canine index as a sex determinant

Teeth are extremely durable even at high temperatures and may be identified even when the rest of the body has undergone decomposition. Of all the teeth in the human dentition, canines are considered to be the most stable teeth. The labiolingual thickness of crown and root and the anchorage in the alveolar process along with the self-cleansing quality, tends to preserve these teeth throughout life. Canines seem to be unaffected by extreme catastrophic events like air crashes or hurricanes.[12,13] The method comprises of evaluating dental casts in the age group of 14-20 years. Boaz *et al*.[13] evaluated the possibility of dimorphism of the canines being used as a valid tool in the forensic and legal identification of an individual. In this study, alginate impressions were taken for each subject and study models were prepared from dental stone. The mesiodistal and buccolingual measurements of mandibular canines were subjected to statistical analysis using the t-test to determine whether significant differences existed between tooth ages in males and females. Their study revealed that the mean values of the buccolingual and mesiodistal dimensions of the mandibular left canine were greater in females than in males and the mean values of the mesiodistal dimensions of the mandibular right canine in females were greater than that in males in the given sample. While in another study done on the western Uttar Pradesh population, Reddy *et al*.[14] measured maximum mesiodistal diameter of mandibular canines using a Vernier caliper. Observed mandibular canine index (MCIo) was calculated as the ratio between the maximum mesiodistal crown width of mandibular canine and canine arc width. With the standard MCIs it was possible to detect sex in the population of western Uttar Pradesh to an extent of about 72% [Figure 1a-b].[14]

Table 1: Milestones in forensic odontology

Year	Milestone
1453	First reported case of dental identification; Earl of Shrewsbury who fell in the battle of Castillon
1775	Dr. Paul Revere: the first forensic odontologist, who identified the remains of a victim based on the retrieval of a prosthesis constructed by him
1831	Leuchs discovered amylase in saliva
1849	The first conviction based on dental evidence of crowns from charred remains of the victim
1898	First treatise on forensic odontoly was written by Dr. Oscar Amoedo the father of Forensic Odontology
1921	Mueller suggested that detection of amylase can be a presumptive test for salivary stains
1929	Ki performed the first comprehensive investigation in identification of isoantibodies in saliva
1932	Locard recommended the use of lip prints in identification
1937	Murder trial conviction based on bite mark evidence first time in history
1946	Welty and Glasgow devised a computerized program to sort 500 dental records
1985	Jeffreys 'discovered' DNA fingerprints

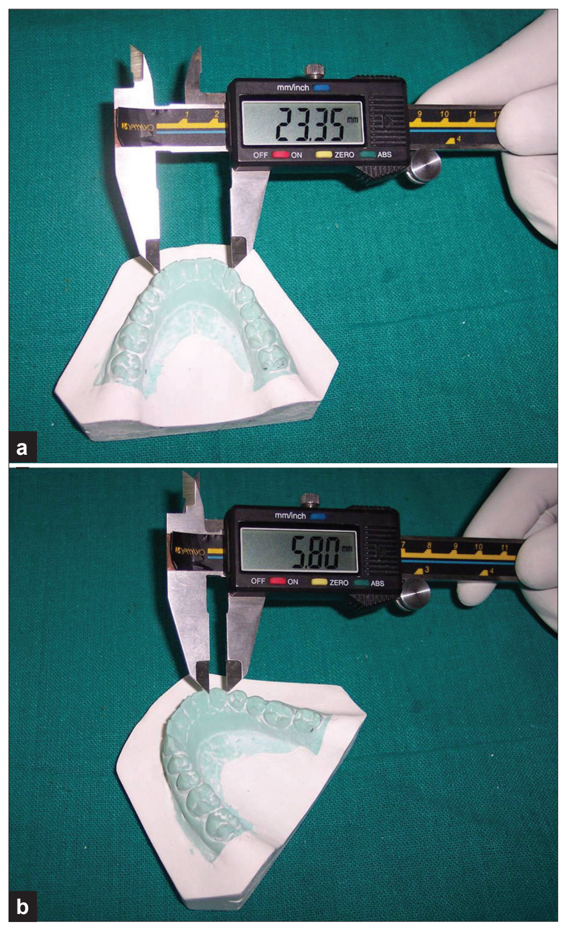

Figure 1: (a) Measuring maximum canine arc width and mesiodistal crown width of mandibular canine. (Courtesy Reddy *et al.* Ref no. 14); (b) Measuring maximum canine arc width and mesiodistal crown width of mandibular canine. (Courtesy Reddy *et al.* Ref no. 14)

Cementum annulations for age estimation

Recent research indicates that the tooth cementum annulations may be used more reliably than any other morphological or histological traits of the adult skeleton for age estimation.[15]

In this technique, the area at the junction of apical and middle third of the root is studied on photomicrograph. The alternate light and dark bands are counted on the photo with the help of image analysis software and added to the average eruption time of individual tooth. The predicted age of the individual is thus obtained as:

Number of incremental lines (n) = X/Y

Where, X is the total width of cementum from dentinocemental junction to cementum surface and Y is the width of cementum between the two incremental lines.

By adding average age of eruption in years for each tooth as presented in Gray's Anatomy[16] in the counted number of incremental lines, the chronological age of the individual was obtained.

E = n + t

where, estimated age = number of incremental lines (n) + eruption age of tooth (t).[17]

Enamel Rod End Patterns

Tooth prints are enamel rod end patterns on the tooth surface. These patterns are unique to an individual tooth of the same individual and in different individuals. Physical or biological measurement pertaining to an individual is termed biometrics, which are being automated to eliminate the need for human verification, and a number of new biometric measurements have been developed, tapping advantage of improved understanding of the human body and advanced sensing techniques. Manjunath *et al* [6,18] have classified automated biometrics into two main types: verification and identification. In verification (one-to-one comparison), the biometric information of an individual, who claims certain identity, is compared with the biometric information of that particular individual in the database. The acceptance or rejection of the identity of an individual can be determined by the comparative results. While in identification (one-to-one comparison) the biometric information obtained is compared with information of a group of individuals that is stored on the database. To uniquely identify an individual based on biometric data it should be:[6,18]

- Highly unique to each individual
- Easily obtainable
- Time-variant (should not undergo any significant change over a period of time)
- Easily transmittable, able to be acquired as nonintrusively as possible
- Distinguishable by humans without much special training.

Enamel rods are laid down by the ameloblasts in an undulating and intertwining path. This is manifested on the outer surface of enamel as patterns of the ends of a series of adjacent enamel rods. These patterns on the enamel surface are called as tooth prints.[6,18] Each tooth print is composed of combination of eight different subpatterns, and each tooth print is unique to a particular tooth. This uniqueness of the toothprint is used as a valuable tool in forensic science for personal identification. Advantage of the technique is that three successive tooth prints of each tooth show a similar pattern of enamel rod ends and similar distribution of minutae points. However, inspite of standardization, there can be variation, as inclusion or deletion of even a single cluster of enamel rods, could lead to variation in the *minutae* score.[6,18]

Manjunath *et al*[6] coined the term *ameloglyphics* (*amelo* meaning enamel; *glyphics* meaning carvings) – for the study of enamel rod patterns on tooth surface. These enamel rod end patterns can be duplicated by various methods like acetate peel technique, rubber base impression, etc. In the acetate peel technique a circle of 5-mm diameter is drawn on middle third of the labial surface. The labial surface is ground except the circular area. Ungrounded circular area is etched with 10% orthophosphoric acid for 20 seconds, washed with water and dried. Thin layer of acetone is applied over a piece of cellulose acetate film and placed over etched surface for 20 minutes. The principle of the technique is that acetone dissolves a layer of cellulose acetate and the dissolute settles down along the irregularities on the enamel surface. After 20 minutes, the film is gently peeled off followed by observation under light microscope. Photomicrograph of acetate peel at ×40 magnification is subjected to biometric analysis using verifinger software. The software uses certain points called *minutae* for identification and comparison. *Minutae* are defined as discontinuities of lines, line endings, dots, very small lines, ponds and/or empty spaces between two lines.[6,18]

The age-related changes in the dentition can be divided into three categories: formative, degenerative and histological. Formative changes can be good predictors till the age 12. They include the completion of the crown, eruption of the crown into oral cavity and completion of the root. Degenerative changes include attrition, periodontosis, secondary dentin and cementum apposition (both seen microscopically), root resorption and transparency of the root seen in ground sections.[19]

Amino acid racemization studies are also used to determine age. Aspartic acid has been reported to have the highest racemization rate of all amino acids and to be stored during aging. In particular, L-aspartic acids are converted to D-aspartic acids and thus the levels of D-aspartic acid in human enamel, dentine and cementum increase with age. The D/L ratio has been shown to be highly correlated with age.[11]

Aspartic acid is most commonly used based on its presence in human dentin. Aspartic acid exhibits optical phenomena by existing in the dextro (D) or laevo (L) forms. L-aspartic acid is found in human dentin and with time converts into the D-aspartic acid. It has a slow metabolic turnover and therefore is slow to decompose. Thus, assessment of the D/L ratios in dentin, by chromatographic techniques, can be correlated to age. During the last decade aspartic acid racemization rate is used widely in chronological age determination and seems to be the method of choice for the next years.[11]

Dentin Translucency

Dentinal translucency is one of the morpho-histological parameters considered best for dental age estimation, not only in terms of accuracy but also simplicity. By convention, translucency has been measured using calipers.[20,21] Acharya (2010)[22] described a method to measure translucency on sectioned teeth using commercially available computer hardware and software. However, attempts to quantify translucency using digital aids have been proposed over the last 2 decades. These methods require capturing tooth images on a video camera, converting the analog signal to digital and subsequent image processing using customized software programs.[22] With advances in computing technology, digital evaluation of translucency can be more easily accomplished today. The superior results using the digital method are attributed to refined measurements obtained under magnification and the "touch-free" approach of measuring translucency on digital images of thin tooth sections.[22] Valenzuela *et al* (2002)[23] concluded in their study that computer-based translucency measurements contributed best to age estimation.

Panoramic Radiography

Radiography being a non-destructive method also plays a vital role in forensic dentistry to uncover the hidden facts which cannot be seen by means of physical examination. Dental examination and comparison between ante-mortem and postmortem dental records and radiographs produce results with a high degree of reliability and relative simplicity. Panoramic radiographs are also helpful to determine the age of the individual by assessing the stage of eruption.[24,25]

The use of radiographs is characteristic of techniques that involve observation of morphologically distinct stages of mineralization. Age estimations are also based on the degree of formation of root and crown structures, the stage of eruption and the intermixture of primary and adult dentitions.[26]

The size of dental pulp cavity is reduced as a result of secondary dentin deposit. The measurements of this reduction can be used as an indicator of age (Kvaal *et al*, 1995).[27] Using radiographs, the pulp length and width are measured. These ratios are found to be significantly correlated with age. Results show the strongest correlation with age to be in ratio between the width of the pulp and the root. This indicates that the rate of deposition of dentine on the mesial and distal walls is more closely related to age than that on the roof of the pulp cavity. However, the limitation of the technique is that the correlation between age and the ratios between pulp and the root length was found to be significant for only maxillary cuspids and premolars.[24,27]

Bite Marks in Forensic Dentistry

Bite marks are an important and sometimes controversial aspect of forensic odontology. Although there are many cases in which bite mark evidence has been critical to the conviction or exoneration of criminal defendants, there is continuing dispute over its interpretation and analysis.[28] For bites on human skin, a potential bite injury must be recognized early, as the clarity and shape of the mark may change in a relatively short time in both living and dead victims. Bite marks most often appear as elliptical or round areas of contusion or abrasion, occasionally with associated indentations.[28]

Once the mark is initially evaluated, it should be examined by a forensic odontologist to determine if the dimensions and configuration are within human ranges. Since a large proportion of individuals (80-90%) secrete the ABO blood groups in their saliva, swabbing the area and a control area elsewhere on the body should be completed before the body is washed.[29] Although there have been descriptions of using fingerprint dusting methods, photography is the primary means of recording and preserving the bite mark and is critically important in documenting the evidence.[30] When there are indentations in the skin, or to preserve the three-dimensional nature of the bitten area, impressions should be taken to fabricate stone models. This is done by fabricating custom impression trays and taking an impression of the mark and surrounding skin with standard dental impression material. These impressions are then poured in dental stone to produce models.[31,32] After the initial analysis is complete, there may be a need to preserve the actual skin bearing the mark. A ring of custom tray material can be made to fit like a hoop, closely approximating the skin, which can then be attached to the skin with cyanoacrylate adhesive and stabilized with sutures. When the pathologist completes the autopsy, the bite mark can be excised with the supporting framework in place.[33]

Although there are questions about the scientific merit of some aspects of the evaluation process of bite mark evidence, numerous legal precedents allow for the admissibility of such evidence.

When reporting on bite mark evidence, dentists should freely admit the inherent obstacles to accurate analysis and apply the bite mark evidence in a manner consistent with scientific principles.[28]

Cheiloscopy and Palatoscopy

The theory of uniqueness is a strong point used in the analysis of fingerprints and bitemarks to convince the court of law. Likewise, even lip prints and palatal rugae patterns are considered to be unique to an individual and hence hold the potential for identification of an individual. Cheiloscopy (from the Greek words *cheilos* - lips, *skopein* - see) is applicable mostly in identifying the living, since lip prints are usually left at crime scenes and can provide a direct link to the suspect. Lip print studies are unique to one person, except in monozygotic twins.[34] Like fingerprints, lip grooves are permanent and unchangeable. It is possible to identify lip prints as early as the sixth week of intrauterine life. The junction of the lip, skin and mucosa is formed by a white wavy line called the labial cord. The mucosal area, Klein's zone, is covered with wrinkles and grooves forming a characteristic pattern- the lip print. Lip anatomy is also analyzed i.e., their thickness and position. Caldas *et al*.[34] analyzing the anatomical aspects i.e., thickness and position of lips, have stated that lips can be horizontal, elevated or depressed. Lip thickness also varies according to the race e.g., thin lips (common in the Europian Caucasian), medium (from 8 to 10 mm, are the most common type), thick or very thick lips (usually having an inversion of the lip cord and are usually seen in African Americans).[34] Labeling of a particular pattern is based on the numerical superiority of types of lines present that is vertical, intersected, branched or reticular. If more than one pattern predominates it is termed as undetermined [Table 2].[35,36]

The sex of the individual was determined as:[37,38]

Type I, I' and Type II patterns were dominant in females. Type III and Type IV patterns were found predominantly in males [Figure 2].

Techniques for Rugoscopy

Palatal rugae, also called *plica palatinae* transverse and *rugae palatine*, refer to the ridges on the anterior part of the palatal mucosa, each side of the median palatal raphae and behind the incisive papilla. Catastrophic accidents involving plane crashes, fires and explosions can destroy the fingerprints but, interestingly, palatal rugae patterns are preserved. Once formed they do not undergo any changes except in length, due to normal growth, remaining in the same position throughout an entire person's life.[34]

Stereoscopy is a technique in which 3D image of palatal rugae anatomy is obtained, based on the analysis of pictures taken with the same camera, from two different points, using special equipment. Stereophotogrammetry, by using a special device called *Traster Marker*, allows for an accurate determination of the length and position of every single palatal ruga. While the overlay print of palatal rugae in a maxillary cast is termed as calcorrugoscopy [Figure 3].[39]

The rugae pattern is classified based on their length, shape, direction and unification, proposed by Lysell (1955)[40] and later modified by Thomas and Kotze (1983) [Table 3].[41]

Caldas *et al*[34] have cited studies where it has been suggested, that generally, there is no bilateral symmetry in the number of primary rugae or in their distribution from the midline. It has been observed that there are slightly more rugae in males and on the left side in both genders.[34]

While lip prints behold the potential for the sex identification of an individual, the same does not hold true for the palatal rugae patterns. In a comparative study of cheiloscopy versus palatoscopy for the sex identification, a statistically significant difference was found between males and females for the lip prints, while no significant difference was found between them for the rugae patterns.[42]

DNA in Forensic Odontology

Forensic science is ever in pursuit of the perfect tool in human identification. The identification of highly variable regions of DNA with unique patterns for each individual, DNA finger printing became a byword in forensics. Several biological materials may be employed for isolation of DNA and accomplishment of laboratory tests for human identification, including teeth, bone tissue, hair bulb, biopsy sample, saliva, blood and other body tissues.[8,11]

Most varied techniques were applied for identification of thousands of victims of South Asian Tsunami disaster of

Table 2: Classification schemes in cheiloscopy

Classification	Criteria
Santos (1967)	Straight line
	Curved line
	Angles line
	Sine-shaped curve
Susuki and Tsuchihasi (1970)	Type I; clear cut grooves running vertically across lips
	Type I': partial length of Type I grooves
	Type II: branched grooves
	Type III: intersected grooves
	Type IV: reticular pattern
	Type V: other pattern

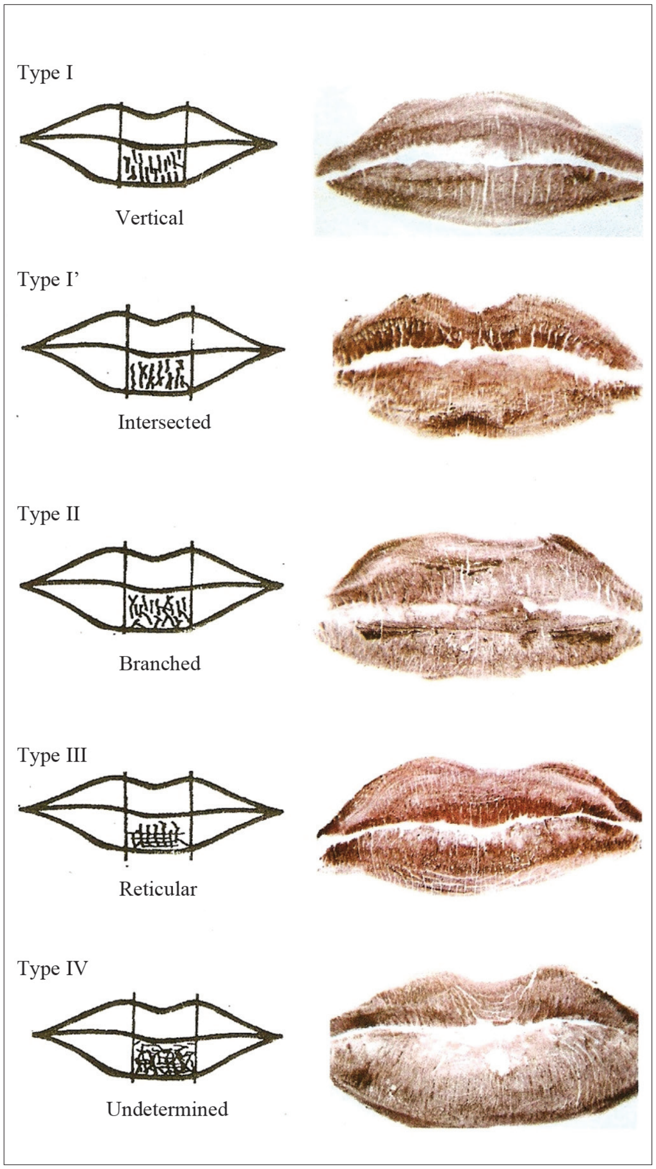

Figure 2: Patterns of lip prints (Courtesy of Dr. Sivapathasundharam B, Dr. Ajayprakash P, and Dr. Sivakumar G, Lip prints (cheiloscopy) Indian J Dent Res, 10:234-37, 2001)[45]

Figure 3: Rugae patterns analyzed on cast

2004, such as forensic pathology, forensic dentistry, DNA profiling and fingerprinting. The teeth are an excellent source of genomic DNA because PCR analyses allow comparing the collected post-mortem samples to known ante-mortem samples or parental DNA. Silva *et al*[9] have emphasized that mitochondrial DNA is another type of material that can be used for body identification. Its main advantage is the high number of copies per cell when the extracted DNA samples are too small or degraded, such as those obtained from skeletonized tissues, the likelihood of obtaining a DNA profile from mitochondrial DNA is higher than that with genomic DNA. Saliva is also a feasible source of DNA for forensic purposes. Saliva is deposited usually through bite marks in homicides, assaults, child abuse or other criminal cases. Saliva can also be retrieved from cigarette butts, postage stamps, envelops, clothes, skin, etc. Effective collection of dried saliva has been achieved by the double swab technique (using a wet cotton swab followed by a dry cotton swab on surface with dried saliva).[8,11]

Sex Determination from Pulpal Tissue

The sex determination from pulpal tissue depends on the presence or absence of X-chromosome. Barr body is an intranuclear mass usually lying against the nuclear membrane in the females. Sex determination from human tooth pulp in cadavers is possible up to a period of 4 weeks. But teeth can also serve as an excellent source of genetic material.[8,9] Non-restored teeth are more appropriate for DNA analysis than restored teeth. Molars are preferred over anterior teeth. Small amounts of DNA are relatively well-preserved in fossils, buried mummies and various remnants of human dental tissues. The procedure consists of cryogenic grinding of pulverized teeth under sterile conditions and in the presence of liquid nitrogen can yield enough material for DNA analysis. PCR enables the amplification of very tiny amounts of DNA to concentrations suitable for diagnostic analyses.[8,9] Jeffreys, *et al* (1985)[43] created radioactive molecular probes that could recognize highly variable regions of DNA and thus determine the specific patterns

Table 3: Thomas and Kotze (1983) classification of palatal rugae

Criteria
Length
Primary rugae
A–5 to 10mm
B–10 mm or more
Secondary rugae: 3-5mm
Shape
Fragmentary rugae: less than 3mm
Curved
Wavy
Straight
Circular

of each individual, which were named DNA fingerprints. Silva *et al* [9] in their review study, have emphasized the high reliability of currently performed DNA profile tests. They have affirmed that these tests can be used as legal proofs in human identification.

Summary and Conclusions

The concept of fusing dental evidence in forensic investigation has kindled so much interest in the recent past that forensic odontology is even suggested as the single positive identification method to solve certain forensic cases. A forensic dentist is concerned with the handling and collation of dental evidence and assists law enforcement agencies in the detection and resolution of criminal and civil proceedings. The fact that physiological variations, pathoses and effects of therapy of dental hard and soft tissues are unique to every individual forms the basis of this branch of medical science.[1,2,11]

The contribution of a forensic odontologist in medicolegal proceedings cannot be overstressed. In spite of having progressed by leaps and bounds through various techniques in the identification of accident victims and in investigative criminology, forensic odontology is still bound by precincts.[8] Humanitarian aims pursued by volunteering dentists during mass disasters should be refrained to assure quality work and high forensic standards. Guidelines and international principles and codes are needed especially when working in an international arena. This would allow disaster victim identification (DVI) teams from different nationalities to work in a synergic and universal way. Strategies for developing an international forensic odontology capacity and resources are needed for the management of dead bodies following a mass disaster, together with universal guidelines and codes. Interpol's forms could be a good and readymade starting point to meeting these requirements. These DVI forms with a graphic interface will ease data and dental data display and will be usable anywhere in the world, on any computer and also allowing simultaneous different disasters recording.[44]

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